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Fibrin generation during the diabetic pregnancy

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Complications of the diabetic pregnancy include an increased risk of maternal hypertension and sudden, unexplained fetal death. Each may be associated with an increased tendency for thrombosis. Abnormalities of both soluble coagulation components and platelet function are present in diabetic patients with advanced vascular disease. These alterations support the concept of a hypercoagulable state. Endothelial cells from diabetic patients release increased quantities of von WILLEBRAND's factor [8, 12]. Both platelet adhesion and aggregability are increased; metabolism is altered. The increased adhesiveness may be secondary to fibrin coating of the platelet [17, 10]. Fibrinogen is frequently elevated; fibrinolysis is depressed [8]. Antithrombin III, the principle in vivo inhibitor of thrombin generation, has variously been reported as increased, decreased, or unchanged in patients with diabetes [1, 2, 9]. The discrepancy may be secondary to differences in the degree of vascular disease. Indeed, increased antithrombin III activity may be an early compensatory response to increased fibrin generation [8]. Some investigators have reported improvement of these coagulation parameters after sustained periods of euglycemia [18, 7, 13, 5, 6]. Whether some or all of these abnormalities are present in the diabetic patient without advanced vascular disease is unclear [3]. Further, it is difficult to distinguish whether these coagulation abnormalities are the direct result of hyperglycemia or secondary to an indirect effect upon the vascular endothelial cell.

Measurement of fibrin turnover is one way of documenting hypercoagulability. Fibrin catabolism has not previously been examined in the diabetic pregnancy. Fibrinopeptide A is the first peptide cleaved from fibrinogen during thrombin-mediated fibrin generation [15]. With a three minute half-life, FPA concentration reflects current fibrin generation. We have previously demonstrated that the level of FPA increases during normal pregnancy with the advancement of gestational age [19]. The findings of the current study suggest that fibrin catabolism is further increased in the pregnant diabetic patient despite aggressive blood glucose control.

1 Materials and methods

As part of an investigation into coagulation abnormalities during pregnancy, FPA was prospectively and longitudinally determined by radioimmunoassay in 20 pregnant, diabetic women between 26 and 38 weeks' gestational age. Women in WHITE's classes B–F received insulin two to four times per day and performed home monitoring. The insulin adjustments were based on preprandial measurements; these were unavailable for our analyses. The overall diabetic control was determined by total hemoglobin A_1 (HbA_1) and fasting plasma glucose levels which were available from the patient records. Total HbA_1 was measured

using the CORNING electrophoretic method. Plasma glucose was determined with a BECKMAN glucose analyzer. All determinations included were computed from individual patient means.

Fibrinopeptide A was measured by an investigational, double antibody radioimmunoassay kit developed by MALLINCKRODT, Inc., St. Louis, Missouri. All assays were performed using a single production lot. This assay is now commercially available. Early investigation demonstrated that paired samples from the opposite arms of volunteers using vacutainer and syringe yielded similar measurements. Plasma was prepared by centrifugation in a tabletop centrifuge at 1800 x g for 20 minutes at room temperature. The resulting supernatant was stored at $-40\,^{\circ}C$ until assayed. Reproducibility was excellent. Intra-assay and interassay variations for FPA in the laboratory were 2% and 4% respectively. A single AT III determination at 36 weeks' gestation was available in some patients (N = 15). Antithrombin III activity was determined by the method of ODEGAARD [16]. The mean FPA concentration of the diabetic women at each gestational age was compared using the WILCOXAN Rank Sum Test, to the corresponding value obtained from 17 nondiabetic women sampled longitudinally during the study interval [19]. Statistical significance was assumed if $p \leqslant 0.05$.

2 Results

Patient age ranged from 23 to 36 years (mean = 28 ± 4.2 years) (Tab. I). Fourteen women were WHITE's class A–C. Good diabetic control for the group overall is indicated by near normal HgA_1 and fasting plasma glucose values. The mean hemoglobin A_1 ± 1 SD was 7.1% ± 1.2% (N = 54 samples, normal range 4.6–7.0%). The mean fasting plasma glucose concentration ± 1 SD was 101.9 mg% ± 21.5 mg% (N = 240 samples).

Tab. I. Demographic characteristics

WHITE's Class (N)	Mean Age ± 1 SD
A (2)	32
B (9)	28.5 ± 4.5
C (3)	25.7 ± 2.9
D, F, R (6)	27.5 ± 4.2
* Mean Total HbA_1	7.1 ± 1.2
** Mean Fasting Plasma Glucose	101.9 ± 21.5 mg%

* mean of 18 individual patient means
** mean of 20 individual patient means

Tab. II. Mean fibrinopeptide A in diabetic women during pregnancy

G.A.	Normal FPA ηg/ml ± 1 SD	Diabetes (N) FPA ηg/ml ± 1 SD
26 weeks	3.3 ± 0.7	3.9 ± 1.4 (12)
28 weeks	3.9 ± 0.3	*7.0 ± 4.2 (9)
30 weeks	4.1 ± 2.1	*5.8 ± 2.1 (13)
32 weeks	4.7 ± 1.2	4.9 ± 1.9 (10)
34 weeks	4.3 ± 1.3	5.7 ± 2.8 (13)
36 weeks	4.6 ± 1.9	*6.5 ± 2.8 (14)
38 weeks	4.5 ± 1.6	*9.5 ± 5.5 (6)

* $p < 0.05$

Mean FPA for the diabetic women exceeded control values at each gestational period. Despite large standard deviations, significant differences were found in four of the seven intervals (Tab. II). While the highest FPA value was noted in a patient with advanced diabetic vasculopathy, exclusion of this patient did not alter the overall findings. Antithrombin III activity at 36 weeks was similar to control (91.3 vs. 82.3, $p > 0.05$).

3 Discussion

The present data suggest that fibrinogen catabolism, as reflected by the level of FPA, was elevated above normal pregnancy in women with diabetes mellitus. Interpretation of these findings should be cautious in light of the large standard deviations and the sampling errors. Not all patients were sampled at each gestational epoch. Because these women were involved in a study not designed to evaluate abnormalities of coagulation specifically secondary to diabetes, correlations with control and WHITE's class would not be valid. Nevertheless, the findings were striking and suggested the need for a prospective study designed to account for WHITE's classification of diabetes and the degree of glucose control.

In light of the known coagulation abnormalities present in nonpregnant diabetic patients, our

findings are not altogether surprising. However, the possible causes and the effects of accelerated fibrinogen catabolism during pregnancy upon the mother and fetus should be considered. We have previously supported the concept of a hypercoagulable milieu during normal pregnancy by demonstrating elevated FPA levels [19]. Complications of the diabetic pregnancy include an increased risk of hypertension in the mother, and sudden, unexplained fetal loss. Each has been associated with an increased tendency for thrombosis.

It is possible that the hypercoagulability of pregnancy is enhanced by the diabetic disease process, and accounts at least in part for the elevated FPA concentrations detected. Reduced prostacyclin production by the umbilical arteries during **in vitro** stimulation of infants born to diabetic mothers has been reported. If such a decrease occurs on the maternal side, the reduced prostacyclin/thromboxane A_2 ratio could promote thrombogenesis and fibrin generation [14]. Poor glucose control might also contribute since large excursions of plasma glucose results in excessive free water movement to and from the intravascular space producing blood flow turbulence at arterial branch points and disruption of the endothelial layer [4].

Another possible explanation of the high FPA observed in the diabetic pregnancy involves the pregnancy-related increase in blood flow to various organs. Elevated plasma beta-thromboglobulin and platelet factor 4, indicative of platelet consumption, have been reported in patients with such vascular diseases as coronary artery disease, peripheral vascular disease, and diabetic angiopathy [10]. Normal blood flow turbulence is increased at the arteriolar branch points and can lead to endothelial cell damage, platelet activation, and subsequent fibrin generation [8]. This turbulence is further increased at sites of atherosclerotic disease. Therefore, increased blood flow during pregnancy to organs with asymptomatic vascular disease, such as the kidney, could contribute to an elevated FPA. An elevated FPA in a pregnant diabetic woman, despite euglycemic control, might identify her as likely to develop clinically evident diabetic angiopathy in the near future. Such information would be of value. If euglycemia decreases the rate at which angiopathy progresses, these patients would benefit from longterm, intensive glucose control.

In summary, a preliminary investigation of FPA in pregnant diabetic women suggests that fibrinogen catabolism is enhanced. Further, this enhancement occurred despite blood glucose levels which approximated normal range.

Summary

Fibrin catabolism was measured during the pregnancy of insulin-dependent diabetic women in both a longitudinal and cross sectional fashion. Samples of maternal peripheral venous blood were obtained in 20 pregnant diabetic women between 26 and 38 weeks' gestational age. Fibrinopeptide A, the first peptide cleaved from fibrinogen during thrombin-mediated catabolism, was measured by radioimmunoassay. Intra-assay and interassay variation for fibrinopeptide A in this laboratory were 2% and 4% respectively. Antithrombin III activity was determined by the method of ODEGAARD. The patients ranged from 23 to 36 years. Overall blood glucose control was good as reflected in near-normal HbA_1 fasting plasma glucose values. The mean $HbA_1 \pm 1$ standard deviation was 7.1% ± 1.2%. The mean fasting plasma glucose concentration was 101.9 mg% ± 21.5 mg%. Mean FPA for the diabetic women exceeded control values at each gestational period. Significant differences were found in four of the seven intervals. While the highest FPA was noted in a patient with advanced diabetic vasculopathy, exclusion of this patient did not alter the overall findings. The findings were striking and suggest the need for a prospective study designed to account for WHITE's classification of diabetes and the degree of glucose control. Because complications of the diabetic pregnancy include an increased risk of hypertension in the mother and sudden, unexplained fetal loss, two complications associated with abnormal clotting, the increase in fibrin catabolism in patients in tight metabolic control would suggest that events other than glucose regulation impact upon fibrin catabolism and possibly pregnancy outcome in the diabetic mother.

Keywords: Diabetes, fibrinopeptide A.

Zusammenfassung

Fibrinbildung in diabetischen Schwangerschaften
Wir untersuchten den Fibrinstoffwechsel bei insulinpflichtigen diabetischen Schwangeren sowohl in Longitudinal- als auch in Querschnittsstudien. Dazu wurde bei 20 Diabetikerinnen zwischen der 26. und 38. Schwangerschaftswoche peripheres Venenblut abgenommen. Das Fibrinopeptid A (FPA), welches als erstes Peptid während des thrombinvermittelten Prozesses vom Fibrinogen abgespalten wird, wurde radioimmunologisch bestimmt. In unserem Labor lag die Streuung für einen bzw. unterschiedliche Ansätze bei 2 % bzw. 4 %. Die Antithrombin-III-Aktivität wurde nach der Methode von ODEGAARD bestimmt. Das Alter der Patientinnen lag zwischen 23 und 36 Jahren. Wie das HbA_1 und die Nüchternblutzucker-Spiegel zeigen, waren die Diabetikerinnen gut eingestellt: das HbA_1 betrug im Mittel 7,1 % ± 1,2 %, der Nüchternblutzucker 101,9 mg % ± 21,5 mg %. Der FPA-Spiegel bei diabetischen Schwangeren lag zu jedem Zeitpunkt der Schwangerschaft oberhalb der Vergleichskontrolle. Dabei waren in 4 von 7 Zeitintervallen die Unterschiede statistisch signifikant. Die höchsten FPA-Spiegel wurden bei einer Patientin mit fortgeschrittener diabetischer Vaskulopathie gefunden. Auch wenn man diese Werte herausnahm, änderte sich das Gesamtergebnis nicht. Die Ergebnisse waren beeindruckend und legen nahe, eine prospektive Studie unter Berücksichtigung der Diabetes-Klassifikation nach WHITE und der Abstufung der Blutzuckerkontrollen durchzuführen. Bei diabetischen Schwangerschaften besteht ein erhöhtes Risiko für einen Hypertonus der Mutter sowie für eine Fehlgeburt, wobei beide Komplikationen mit einer abnormen Gerinnung assoziiert sind. Ein erhöhter Fibrinkatabolismus bei Patientinnen mit strenger Stoffwechselführung deutet darauf hin, daß neben der Glukoseregulation andere Mechanismen auf den Fibrinstoffwechsel und den Schwangerschaftsverlauf bei diabetischen Patientinnen einwirken.

Schlüsselwörter: Diabetes, Fibrinopeptid A.

Résumé

Production de fibrine au cours de la grossesse chez la diabétique
On a mesuré le catabolisme de la fibrine au cours de la grossesse de femmes diabétiques insulino-dépendantes, à la fois de façon longitudinale et à la fois en sections croisées. Chez 20 diabétiques enceintes entre 26 et 38 semaines d'âge gestationnel, on a obtenu des prélèvements de sang veineux périphérique maternel. Le fibrinopeptide A, premier peptide coupé du fibrinogène au cours du catabolisme cédiaté par la thrombine, a été dosé par méthode radio-immunologique. Les variations de ce laboratoire pour les dosages entre eux ou pour un même dosage sont de 4 % et de 2 % respectivement. On a mesuré l'activité d'antithrombine III par la méthode d'ODEGAAD. Les patientes étaient âgées de 23 à 36 ans. Le contrôle global de la glycémie était bon avec des valeurs d'HbA_1 proches de la normale ainsi que des valeurs de la glycémie à jeûn. Les valeurs moyennes d'HbA_1 ± 1 déviation standard étaient de 7,1 % ± 1,2 %. La moyenne des glycémies à jeûn était de 101,9 mg % ± 21,5 mg %. La moyenne des FPA chez les diabétiques dépassait les valeurs témoins pour chaque période gestationnelle. On a trouvé des différences significatives pour 4 des 7 intervalles. Bien que la plus haute valeur de FPA ait été trouvée chez une patiente présentant une vasculopathie diabétique avancée, l'exclusion de cette patiente n'a pas altéré les données globales. Les données sont éclatantes et suggèrent la nécessité d'une étude prospective destinée a vérifier la classification des diabètes de WHITE et le degré du contrôle glucidique. En raison des complications de la grossesse de la diabétique comprenant un risque accru d'hypertension maternelle et des morts fœtales brutales et inexpliquées, ces deux complications s'accompagnent d'une coagulation anormale, l'augmentation du catabolisme de la fibrine, chez des patientes avec un étroit contrôle métabolique suggèrerait que des facteurs autres que la régulation glucidique agissent sur le catabolisme de la fibrine et peut-être sur l'évolution de la grossesse des mères diabétiques.

Mots-clés: Diabète, fibrinopeptide A.

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